Screening of Iron Nanoparticle Forming Bacteria from Radiation Resistant Microorganism Library to Develop Biomaterials for Bioremediation

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1. Introduction

Due to the danger and cost of cleanup of radioactive sites or wastes by physicochemical processes, bioremediation methods are being developed for cleanup of contaminated ground and groundwater. To date, the most developed microbial treatment proposed for high-level radioactive sites employs the radation-resistant bacterium *Deinococcus radiodurans.* However, the use of *Deinococcus* spp. is limited by their sensitivity to the culture condition such as high salt concentration and low pH. Here we isolated several stress-resistant *Deinococcus* species from the radiation-resistant microorganism library to develop the bacteria-based biomaterials for bioremediation.

2. Methods and Results

In this section, we described detailed methods for bacterial growth, stress resistance test, absorption efficiency for iron containing chemical.

2.1 Bacterial strains and culture condition

D. radiodurans R1 (ATCC13939) and other strains were grown aerobically at 30°C in tryptone glucose yeast (TGY) broth (0.5% tryptone, 0.1% glucose, 0.3% yeast extract) with agitation at 200 rpm. Bacterial growth was assessed by measuring optical density (OD) at 600 nm for all strains. Ferric chloride ($FeCl_3$), Ferric citrate and Ferrous sulfate ($FeSO_4$) were purchased from Sigma Aldrich Co. (St Louis, MO, USA). All reagents used were of analytical grade. The ferric(III)/ ferrous(II) stock solutions were obtained by dissolving chemicals in ultrapure water. The $FeCl_3$/$FeSO_4$ solutions used in this study were prepared by dilution of a 100 mM stock solution. The Ferric citrate solution used in this study was prepared by dilution of a 20 mM stock solution.

2.2 Screening of resistant strains against NaCl, NaI, CsCl

D. radiodurans R1 (ATCC13939) and other strains were grown in TGY agar media including 0~0.4 M NaCl, 0~0.4 M NaI, or 0~50 mM CsCl. After inoculating the serially diluted cells on the agar plate, it were incubated in 30°C for 3 days and the resulting colony numbers were counted to compare the resistance for each stress condition among the selected strains.

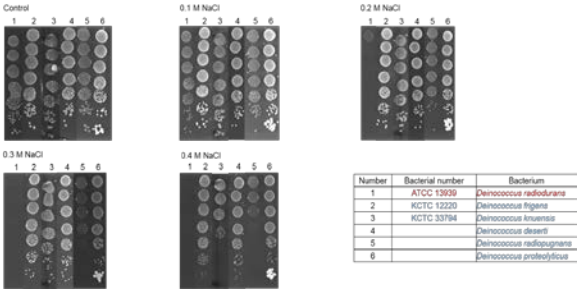

Fig. 1 Isolation of NaCl resistant *Deinococcus* species from the radiation-resistant microorganism bank resource. Among 65 strains, we isolated 5 strains with resistance to over 0.4 M NaCl, in which the representative radiation-resistant bacteria, *D. radiodurans*, cannot survive.

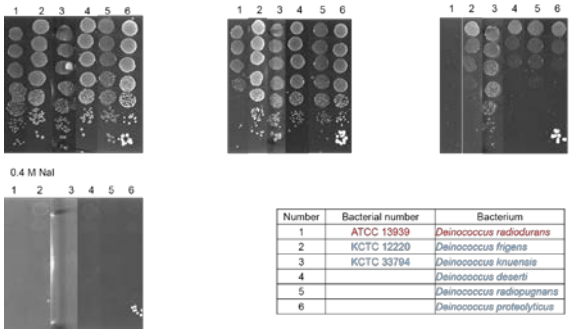

Fig. 2. Isolation of NaI resistant *Deinococcus* species from the radiation-resistant microorganism bank resource. Among 65 strains, we isolated 5 strains with resistance to over 0.2 M NaI, in which condition the representative radiation-resistant bacteria, *D. radiodurans*, cannot survive.

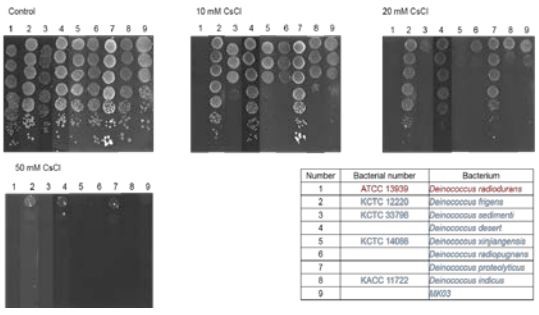

Fig. 3. Isolation of CsCl resistant *Deinococcus*

species from the radiation-resistant microorganism bank resource. Among 65 strains, we isolated 8 strains with resistance to over 10 mM CsCl, in which condition the representative radiation-resistant bacteria, *D. radiodurans*, cannot survive.

2.3 Preparation and Identification of Iron nanoparticles formation

D. radiodurans R1 and other strains were incubated in TGY broth at 30°C until OD600 nm reached 1.0. Then, 1 mM ferric chloride, 1 mM ferric citrate, 1 mM ferrous sulfate were added into cell. The cells were incubated by shaking at 30℃ for 16 hours. 1 ml cell for each condition was harvested, then the cells were washed by PBS at three times. Finally, the cells were resuspended by 200 ul PBS for UV/Vis absorption spectroscopy. The formation of iron nanoparticles in the cell was monitored by the color changes of the reaction mixtures, and the absorption spectrum of the cell was measured at regular intervals (5 nm) by UV/Vis absorption spectroscopy (Varioskan Flash; Thermo Fisher Scientific, MA, USA). The absorption spectra of sample aliquots from 210 to 900 nm were recorded as a function of reaction time.

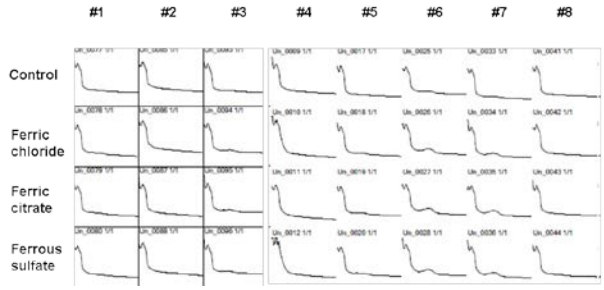

Fig. 4. Absorption spectra of iron compounds in to the Deinococcus species. Absorption spectra were measured in the range of 210~900 nm with 5s interval.

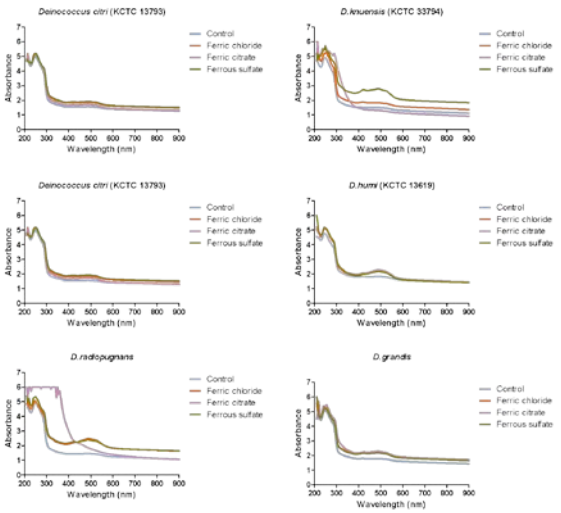

Fig. 5. Selection of 6 candidate strains of *Deinococcus* with the absorption ability in the aerobic condition and further investigation of iron nanoparticle formation inside of the cell.

3. Conclusions

Identification of NaCl, NaI, and CsCl resistant *Deinococcus* species show that there are at least 5 strains for each stress can survive in higher stress condition which may affect the metal nanoparticle biomineralizing experimental condition. Also, we can select 6 strains which show distinguishable absorption spectra compare to *D. radiodurans*. Because the iron nanoparticle formation in bacteria usually conducted in anaerobic condition, our finding can contribute to develop the radiation-resistant bacteria based iron nanoparticle forming biomaterials which can apply for the bioremediation process in the field of decommissioning and decontamination of nuclear facilities.

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